Supporting Information

A Simple and Programmable Dual-mode Aptasensor for

Ultrasensitive Detection of Multidrug-resistant bacteria

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Name	Sequence $(5' \rightarrow 3')$				
MRSA aptamer 1 ^[1]	NH ₂ -CCATCCACACTCCGCAAGGGTGCCCCGGGGGGGCTGTTCAGCGTGGT				
	GGTGGGATGCCGTTTTGGTCCTTAGTCTCCGTCGTCGG <mark>CTGCCTCTACAT</mark>				
	SH-CCATCCACACTCCGCAAGGGTGCCCCGGGGGGGCTGTTCAGCGTGGT				
MRSA aptamer 2	GGTGGGATGCCGTTTTGGTCCTTAGTCTCCGTCGTCGG <mark>CTGCCTCTACAT</mark> -				
	BHQ2				
MRSA probe	TTTTTT <mark>ATGTAGAGGCAG</mark> -Cy5				
<i>E. coli</i> aptamer 1 ^[2]	NH2-GGCAGGACACCGTAACGGGTATGCAGCTATCCCGGGCGCTGTCTGA				
	AGA <mark>TCGTGTGCTGCT</mark>				
E. coli aptamer 2	SH-				
	GGCAGGACACCGTAACGGGTATGCAGCTATCCCGGGCGCTGTCTGAA				
	GA <mark>TCGTGTGCTGCT</mark> -BHQ2				
E. coli probe	TTTTTT <mark>AGCAGCACACGA</mark> -Cy5				
<i>M. tuberculosis</i> aptamer 1 ^[3]	NH2-GGTGTGTTGACTGAGGGGGGGGGGGGGGGGGGGGGGGGG				
M. tuberculosis aptamer 2	SH-GGTGTGTTGACTGAGGGGGGGGGGGGGGGGGGGGGGGGG				
	BHQ2				
M. tuberculosis probe	TTTTTT <mark>GCTATATCCACC</mark> -Cy5				
A.baumanii aptamer 1 ^[4]	NH2-TACATGGTCAACCAAATTCTTGCAAATTCTG <mark>CATTCCTACTGT</mark>				
A.baumanii aptamer 2	SH-TACATGGTCAACCAAATTCTTGCAAATTCTG <mark>CATTCCTACTGT</mark> -BHQ2				
A.baumanii probe	TTTTTT <mark>ACAGTAGGAATG</mark> -Cy5				
P. aeruginosa aptamer 1 ^[5]	NH2-GCGCGCGAGATTAACCCCCCAATGCTGCACCGAGCCACGA				
P. aeruginosa aptamer 2	SH-GCGCGCGAGATTAACCCCCCAATGCTGCACCGAGCCACGA-BHQ2				
P. aeruginosa probe	TTTTTT <mark>TCGTGGCTCGGT</mark> -Cy5				

Table S1. The sequences of the aptamers used in this work.

The highlighted fragment by the yellow background is the binding region of the probe to the aptamer.



Fig. S1. (A) Size distribution histograms of MBs and (B) Magnetic hysteresis loop of MBs and AptMBs.



Fig. S2. Characterization of the synthesized GNPs with sizes of 30, 80, and 120 nm through UV-vis absorption spectra.



Fig S3. TEM image of AptGNPs (scale bar: 500 nm)



Fig. S4. Recognition time of AptGNPs on dual-mode: (A) Mode-DLS and (B) Mode-Flu.



Fig. S5. The average $D_{\rm H}$ of MRSA.



Fig. S6. The hydrodynamic diameter among 10 independent replications in the absence of MRSA. The threshold value of fluorescence intensity was defined as the mean value of negative samples plus 3 standard derivations.



Fig. S7. Levey-Jennings chart-based control for 10 CFU/mL and 10⁴ CFU/mL of MRSA concentrations detected by Mode-DLS. \overline{X}_1 and \overline{X}_2 represent the mean D_H of 10 CFU/mL and 10⁴ CFU/mL, and their corresponding standard deviation is SD₁ and SD₂, respectively.



Fig. S8. The peak fluorescence intensity among 10 independent replications in the absence of MRSA. The threshold value of fluorescence intensity was defined as the mean value of negative samples plus 3 standard derivations.



Fig. S9. Levey-Jennings chart-based control for 10^2 CFU/mL and 10^6 CFU/mL of MRSA concentrations detected by Mode-Flu. \overline{X}_1 and \overline{X}_2 represent the mean fluorescence intensity of 10^2 CFU/mL and 10^6 CFU/mL, and their corresponding standard deviation is SD₁ and SD₂, respectively.

Table S2. Summary of the proposed methods for MDR bacteria detection.

Mathad	Target	Platform	LOD	Detection	Ref.
Method			(CFU/mL)	time (min)	
EXPAR	MDR bacteria	Yes	6.73	70	[6]
RPA and Cas12a	Drug-resistant	No	1	40	[7]
	Salmonella				
Multiplex PCR and CRISPR-	Drug-resistant	No	50	120	[8]
Cas system	A. Baumannii				
Fluorescent microspheres	MRSA	No	110	10	[9]
Modified RPA	Drug-resistant	No	319	60	[10]
	E. coli				
Blue polymeric nanobeads	MRSA	No	2	5	[11]
/	MRSA	No	845	270	[12]
DAPT	MDR bacteria	Yes	4.63	60	This work

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